Polydisperse ethoxylated fatty alcohol surfactants as accelerators of cuticular penetration. 2: Separation of effects on driving force and mobility and reversibility of surfactant action

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Abstract: Polydisperse ethoxylated fatty alcohol (EFA) surfactants can improve the performance of crop protection agents. At the cuticular level they act as accelerators of penetration by increasing the mobility of active ingredients in the cuticle, the barrier properties of which are mainly caused by cuticular waxes. Polydisperse Genapol C-050 (GP C-050, average formula C12.5E5.8) was also found to increase mobility in wax-extracted polymer matrix membranes (MX) of bitter orange and pear, indicating that sorption of surfactants increased segmental mobility of polymethylene chains in cutin and wax. Sorption into MX of the active fraction of GP C-050 from 5g litre^{-1} micellar solutions was in equilibrium in less than 1h after establishing contact. This is almost 100-fold faster than with cuticular membranes (CM). Temperature dependence of solute mobilities in CM was studied in order to measure activation energies (E_D) of diffusion in the presence and absence of aqueous surfactant solutions. Monodisperse fatty alcohol ethoxylates C8E3, C8E4 and C12E6, and (non-surface-active) tributylphosphate decreased E_D of the model compounds WL 110547 and bifenox in *Citrus, Pyrus* and *Stephanotis* CM by more than 100kJ mol^{-1}. This corresponds to 50 to 275-fold increases of mobilities at 15°C. Our data suggest that the decrease in activation energies with the concomitant accelerating effect on mobility contributes considerably to the effects of so-called activator surfactants. High temperature and accelerators act similarly on barrier properties of CM. It is shown that effects of both monodisperse and polydisperse EFA surfactants on solute mobility are reversible and that radiolabelled C12E8 penetrated pear CM rapidly. However, rates of penetration were lowered by excess amounts of WL 110547 and especially phenylurea.

Partition coefficients of seven organic solutes between *Capsicum* fruit cuticles and GP C-050 were very low and, with the exception of methylglucose, smaller than 1. They decreased with lipophilicity and differed about 100-fold. Especially for the lipophilic compounds they were orders of magnitude lower than octanol/water or cuticle/water partition coefficients, which indicates the limited usefulness of these values for an understanding of penetration of active ingredients from formulation residues.

Keywords: adjuvant; cuticular permeability; foliar uptake; partition coefficients; reversibility; surfactant penetration

INTRODUCTION

In plant protection, foliar application of active ingredients (AI) is the most frequent treatment with crop protection agents (CPA) in the field. Since uptake of many actives, especially systemic compounds, is rarely sufficient, CPA often contain additional surfactants, oils, and other additives, which act on different levels during and after foliar application.[1–6] Several attempts have been made to correlate physicochemical properties of active ingredients and additives with foliar penetration.[7–12] For non-volatile, neutral compounds, the basic properties are the size of the molecule and its lipophilicity. Molecular size is characterised by the molecular mass or molar volume and lipophilicity by the octanol/water partition coefficient or water solubility. Using intact leaves it is not possible to identify clearly the relationships between these properties and rates of foliar penetration. This is due to the difficulty

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in controlling the experimental conditions with leaves such that only one parameter limits the actual rates of penetration without other factors, relevant for foliar uptake, interfering. Furthermore, studies often give inconclusive results because the properties may not even be particularly relevant to the performance of the formulation, since the significance of size and lipophilicity of active ingredients may be different in a formulated product. In optimisation of a formulated product the aim is to manipulate the effect of additives like surfactants on these factors.

Emulsifiers such as polydisperse ethoxylated fatty alcohol surfactants are known to increase the efficacy of herbicides.[6,13,14] Rates of penetration can be influenced by the effect of so-called accelerators[5,15] on the barrier properties of the cuticle and/or by effects of all adjuvants on the physical state of the AI on the leaf surface, which determines the driving force for penetration. The effect of molecular size is related to the mobility of organic solutes in cuticular membranes (CM). This is a parameter defined by the barrier properties of CM. Solute mobility depends exponentially on the molar volume of organic solutes[16–18] and, while the absolute values of mobility may differ greatly among species, quite similar orders of (exponential) selectivity have been found for fruit and leaf CM of the several species studied so far.[16,18] We have shown recently[19] that with CM of *Citrus aurantium* L and *Pyrus communis* L, the selectivity of the cuticle changed in the presence of the polydisperse ethoxylated fatty alcohol surfactant Genapol C-050 (GP C-050). The mobilities of large compounds were increased much more than those of smaller ones and, with compounds having a small molar volume ($<100\,cm^3mol^{-1}$), almost no surfactant effect was observed. This shows that, due to the presence of surfactant, the significance of molecular size for mobility is decreased. This effect will occur with all species under comparable surfactant concentrations in the cuticle, although mobilities may differ in their absolute values.[16,18,20]

This paper reports on the action of Genapol surfactants on single processes during cuticular penetration from a formulation residue. We have measured partition coefficients of AI and organic model compounds between cuticle and the surfactant GP C-050 as well as the velocity of octaethylene glycol dodecyl ether (^{14}C-C12E8) penetration which is a constituent of all Genapol C surfactants. These parameters determine the driving force for the penetration of AI from the surfactant residue. The effects of GP C-050 on mobility were studied at different temperatures and compared with those of monodisperse ethoxylated fatty alcohol surfactants and the non-surface-active accelerator tributylphosphate. The role of cuticular waxes was studied by comparing surfactant effects on CM and MX. Reversibility of surfactant effects on solute mobility is also considered. The paper relates to the same series of active ingredients and model compounds that was used in a previous paper.[19]

2 MATERIALS AND METHODS

2.1 Plant material

Astomatous adaxial cuticular membranes of mature leaves of bitter orange (*Citrus aurantium* L), pear (*Pyrus communis* L cv Bartlett), Madagascar jasmine (*Stephanotis floribunda* Brongn), and *Melicoccus bijugatus* Jacq were isolated enzymatically. Bitter orange plants were grown in growth chambers while pear leaves came from trees in an orchard in Bavaria. These species were used previously.[19] *Stephanotis* and *Melicoccus* were grown in a glasshouse and were selected as representative species with crystalline surface wax (*Stephanotis*) or low solute mobility in the cuticle (*Melicoccus*). Isolated cuticles were obtained from these leaves by enzymatic degradation as described previously,[21] air-dried and stored for at least four weeks at about 8 °C prior to use. Cuticle/water partition coefficients of organic solutes were measured with the stronger and easy-to-handle fruit cuticles of green pepper (*Capsicum annuum* L cv. Bell Boy). Partition coefficients are quite similar for different species and cuticles from leaves and fruits do not differ substantially.[22]

Polymer matrix (MX) membranes of *Citrus* and *Pyrus* were obtained from leaf CM by submerging in chloroform at room temperatures for at least one week; on the last day the chloroform was discarded and twice replaced by chloroform+methanol (1+1 by mass) for one hour. This gave extracted cuticular membranes completely devoid of waxes.

2.2 Chemicals

The experiments were carried out with ^{14}C-labelled model compounds (methylglucose (3-*O*-methyl-α-D-glucose), WL 110547 (1-(3-fluoromethylphenyl)-5-phenoxy-1,2,3,4-tetrazole), phenylurea, and active ingredients (cyanazine, chlorfenvinphos, bifenox, permethrin) which vary widely in physicochemical properties. The data reported previously[19] and those relevant for this paper are given in Section 3. In addition, radiolabelled octaethyleneglycol dodecylether (^{14}C-C12E8, 2090 MBq mmol^{-1}, CEA, Grenoble, France) was used. About 50000 dpm/5 µl of C12E8 was applied per cuticle, corresponding to an amount of 0.2 µg. The addition of excess amounts of solute is given in Fig 2.

The fatty alcohol ethoxylates of the Genapol series (Hoechst, Frankfurt, Germany) were polydisperse and of technical grade. That most used in this study, GP C-050, had a range of the number of ethylene oxide (EO) groups of 0 to 15 with a weighted mean number of 5.8, while for GP C-200 the range was 9 to 23 and the mean number was 17. The alkyl distribution was similar for both surfactants with 10 to 18 methylene groups and the average formula are C 12.5E5.8 (GP C-050) and C 12.5E17 (Genapol C-200). Further details can be found in Baur *et al.*[19] The concentration in aqueous solutions was 5 g litre^{-1}. Monodisperse (pure) tetraethyleneglycol monooctylether (C8E4),

triethyleneglycol monooctylether (C8E3), and hexaethyleneglycol dodecylether (C12E6) (all compounds from Fluka, Neu-Ulm, Germany) were used in desorption experiments at concentrations of 25 mM. Experiments with a non-surface-active accelerator, tributylphosphate (TBP), were carried out with a 5 g litre^{-1} emulsion of TBP in 10 g litre^{-1} aqueous phospholipid suspension (PLS) which was prepared by ultrasonication and was stable for the sample periods studied.

2.3 Partition coefficients

Partition coefficients between cuticle and GP C-050 were measured as previously reported for polyethylene glycol 400.[23] The partition coefficients refer to the masses of the cuticle (m_C) and liquids (m_l) according to

$$K_{CW} = \frac{M_C/m_C}{M_l/m_l} \quad (1)$$

where M is the amount of radioactivity (Bq) of the solute in the cuticle (M_C) or the solution (M_t) respectively. The radioactivities of the test compounds in both phases were measured directly after a period of at least 48 h. The radioactive amount in the cuticle M_C was calculated from the difference of total measured radioactivity M_t and radioactivity due to the amount of adhering surfactant. The latter was obtained from the difference in mass before and after the sorption equilibrium period.[23] For these experiments four to 10 replications were used. The exact procedure followed that for the determination of partition coefficients between cuticle and polyethylene glycol 400, described in detail elsewhere.[23]

2.4 Surfactant penetration

Penetration of ^{14}C-C12E8 was studied after applying the radiolabelled substance with or without non-labelled model compounds (methylglucose, phenylurea, WL 110547) to the outer surface of pear cuticles and measuring their appearance in a receiver medium in contact with the inner surface. This is a simulation of foliar uptake (SOFU) where cuticular penetration proceeds from a more-or-less hydrated formulation residue of active ingredients after spraying water has evaporated. The experimental procedure has been described repeatedly and the reader is referred to the literature for details on the method.[19,24] The experiments were carried out under controlled humidity[24] and refer to a temperature of 25 °C.

The amount M_t penetrated at time t was calculated by summation and related to the total amount applied M_o. The time course of penetration was analysed by plotting the logarithm of the relative amount not penetrated ($-\ln(1-M_t/M_o)$) against time. The right-hand ordinate in Fig 2 (and Figs 3–5) shows the fraction (%) penetrated. Ten replications were used in these experiments.

2.5 Mobility of solutes in the cuticle

Mobility of solutes as affected by surfactants and temperature was measured by unilateral desorption from the outer surface (UDOS).[17–19,25] Radiolabelled solutes were sorbed in the cuticle (CM or MX) by application to the inner surface and desorbed from the outer surface into PLS. According to widely differing rate constants of desorption samples were taken at 0.5-h (with MX) to 24-h (with CM) intervals. The details of the method are described elsewhere.[16,19]

The effect of temperature was studied through the range 15 to 36.5 °C and mostly each CM served both as control (desorption with PLS) and as treatment (desorption with C8E4 or GP C-050). This is possible since the effect of increasing temperature up to 35 °C on solute mobility in *Citrus* and *Pyrus* CM is reversible.[17] In one experiment (*Citrus*/Bifenox), desorption with surfactant (C12E6) was started at 35 °C followed by 25 and 15 °C using the same CM. In this case and in experiments with TBP two different sets of CM were used as control (desorption with PLS) and treatment (desorption with C8E4 or C12E6). Due to high rate constants of desorption with tributylphosphate and C12E6, samples were drawn every hour and the experiment was finished within one day, while, in the other experiments, desorption at each temperature was followed for a number of days, with one or two samples per day. The sample number varied between six (*Citrus*/Bifenox/C12E6) and 15 cuticles (*Pyrus*/Bifenox/C8E3). In the range of 15 to 35 °C there is no miscibility gap in the system water–EFA surfactant.[26,27] In all other experiments the temperature was kept at 25(±0.5) °C.

First-order rate constants k^* of desorption were calculated by plotting the relative amount sorbed [$-\ln(1-M_t/M_o)$] versus t. M_o is the total amount contained initially in the sorption compartment of the CM ($t = 0$) and M_t is the amount of radiolabel desorbed from the outer surface at time t following the procedures described previously.[16–19,25]

3 RESULTS AND DISCUSSION

The driving force for cuticular penetration of organic compounds from a formulation residue (C_{fr}) can be described as the product of the cuticle/formulation residue partition coefficient (K_{Cfr}) and the concentration in the residue. This is valid for compounds which do not accumulate in the outer epidermal wall, because they are mobile in the apoplast and taken up into leaf cells and/or distributed within the leaf tissues. Rates of cuticular penetration depend further on the mobility (k^*) in the limiting skin, which is proportional to the diffusion coefficient of the compound.[5] A simplified steady-state flux $\mathcal{J}$ of active ingredients from the surface residue on the cuticle into the leaf can be written as[15]

$$\mathcal{J} = k^* \cdot l_{ls}(K_{Cfr} \cdot C_{fr}) \quad (2)$$

where l_{ls} is the thickness of the limiting skin, a constant

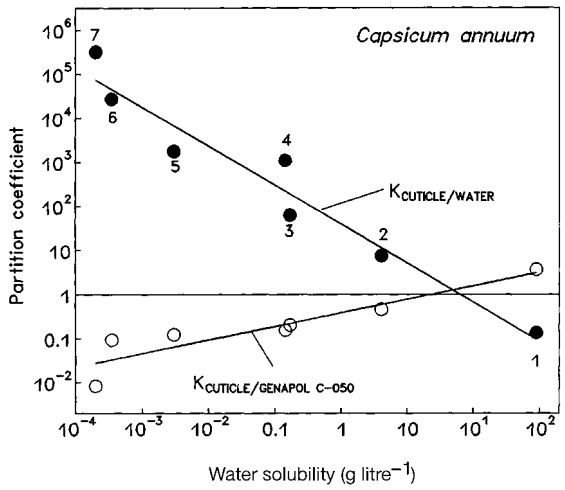

Figure 1. Plot of partition coefficients for the model compounds between green pepper fruit cuticles and (pure) GP C-050 (○) and (●) pepper fruit cuticles and water (taken from Baur *et al*[23]) against water solubilities. Compound identification: **1**, methylglucose; **2**, phenylurea; **3**, cyanazine; **4**, chlorfenvinphos; **5**, WL 110547; **6**, bifenox; **7**, permethrin.

for a given cuticle with a value (about 10^{-6}–10^{-7} m^{-1}) not measurable directly.[15] The equation shows that adjuvants can influence foliar penetration principally by effects on mobility and/or partition coefficient. In the following we will consider how ethoxylated fatty alcohol surfactants and other compounds affect these properties.

3.1 Partition coefficients and driving forces in presence of GP C-050

Uptake into and penetration across the cuticle proceed from the formulation residue. The fraction of active ingredient penetrating the cuticle during evaporation of solvents is usually small in relation to the total amount applied. Therefore solute uptake is proportional to the cuticle/residue partition coefficient (K) and the concentration therein. Figure 1 shows that K was smaller than 1 for all the compounds tested with the exception of the most hydrophilic one, methylglucose. Thus partitioning in the presence of GP C-050 is not in favour of the cuticle. In fact, these partition coefficients show the opposite trend to the octanol (or cuticle)/water partition coefficients, ie they decrease from methylglucose to permethrin (Fig 1). For permethrin the partition coefficients differ almost 10^8-fold. In the double-logarithmic plot of Fig 1 the partition coefficients cuticle/Genapol C-050 increase linearly with water solubility while cuticle/water partition coefficients decrease linearly.

For the same compounds as used in this study we have recently[23] reported partition coefficients between cuticle and polyethylene glycol 400 (PEG400). A comparison with these values (Table 1) shows that for methylglucose, phenylurea and cyanazine, PEG400 was a better solvent than GP C-050, while for the other more lipophilic compounds GP C-050 was superior. For permethrin the partition coefficient between cuticle and GP C-050 is about 100-fold

Table 1. Water solubilities (S_{H_2O}) and partition coefficients between green pepper fruit cuticles and water (K_{CW}), polyethylene glycol 400 (K_{CPEG}), or Genapol C-050 ($K_{CGPC-050}$) of the organic solutes (at 25 °C)

Compound	S_{H_2O} (g litre^{-1})[a]	K_{CW}[a]	K_{CPEG}[a]	$K_{CGPC-050}$
Methylglucose	900	0.13	0.40	3.63
Phenylurea	4.1	7.41	0.25	0.46
Cyanazine	0.17	63.1	0.10	0.20
Chlorfenvinphos	0.145	1096	0.52	0.15
WL 110547	$3\cdot10^{-3}$	1780	0.47	0.12
Bifenox	$0.3\cdot10^{-3}$	30200	0.14	0.09
Permethrin	$0.2\cdot10^{-3}$	323600	1.10	0.01

[a] From Ref 23.

lower than with PEG400. With PEG400, partition coefficients of lipophilic compounds depend on humidity since PEGs are hygroscopic and completely miscible with water.[23] Their solvent power for lipophilic solutes decreases with increasing humidity. Since surfactants like GP C-050 form micelles even in dilute aqueous solution only a slight decrease in the partitioning of lipophilic solutes is expected as long as the surfactant concentration is above the critical micelle concentration (CMC). However, the partition coefficient between cuticle and aqueous (above CMC) GP C-050 on a mass of surfactant basis will be lower, since the solvent action of the ethoxy chain is lost due to hydration in water, while it can contribute to the solvent properties in absence of water.

Partition coefficients cannot be measured for the other Genapol surfactants because the latter are pastelike or solid at ambient temperatures. Concentrated (and hydrated) residues will not differ much from the value of GP C-050 since the higher degree of ethoxylation does not decrease solvent properties for lipophilic compounds, as indicated from the results with pure PEG400. This is suggested also by comparison with cuticle/Tween 80 partition coefficients for bifenox.[28] With a value of $K = 0.75$ with pure Tween 80, this partition coefficient is in the same order of magnitude as the value of 0.11 obtained with the more lipophilic GP C-050 in this study. At K values from about 0.1 to 10 the free energy of sorption ΔG ($\Delta G = -RT \ln K$) at common ambient or leaf-surface temperatures is low,[23,28] and this means that K depends only slightly on temperature. For the lowest cuticle/GP C-050 partition coefficients of permethrin, an increase in temperature of 10 °C is estimated to increase K by about 17%.

The rate of penetration will depend on the affinity of the solute for the surfactant. Two cases can be distinguished. Firstly, if the compound is easily soluble in the surfactant at the amounts applied, the driving force will be rather low due to the low partition coefficients. If the surfactant is not absorbed by the cuticle, the rates of penetration of the AI are then at a maximum at the beginning but will decrease thereafter due to the decrease in concentration in the surfactant residue. This is expected for surfactants like Tween 80

which are not easily taken up into the cuticle. Concentrations and thus driving force can remain high only when the surfactant also penetrates (Section 3.2). In this case the rate of penetration of surfactant should not exceed that of the AI, because otherwise the latter can accumulate on the surface and form a solid residue. Secondly, for compounds which are not easily soluble in the surfactant residue, such as the more polar compounds methylglucose and phenylurea (Section 3.2), precipitation on the leaf surface is possible in spite of the addition of surfactants. Dissolution of the compounds under the given conditions of humidity and temperature is limiting the rate of penetration, although the arguments as given in the first situation are still valid. The addition of humectants or liquid and/or hydrated surfactants which do not penetrate into the cuticle rapidly but form a liquid film on the surface should be beneficial under these conditions.

Attempts have been made to correlate lipophilicity of solutes as measured by octanol/water partition coefficients with rates of penetration both in presence and absence of surfactants.[7–12] While with some species or compounds there was a slight correlation, most results showed no clear dependence of rates of penetration on lipophilicity. The above results for partition coefficients between cuticle and GP C-050 and other surfactants and glycolethers[23,28] clearly show the complexities that occur and that simple correlations could not have been expected. Instead, penetration under steady-state conditions will depend on the amount of surfactant and on the cuticle/formulation residue partition coefficient, which may change with time. If no surfactant was added in experiments with leaves, the solution containing the AI was often a mixture of water and solvents like acetone. Here again the partition coefficient between cuticle and the solution is much lower and differs less among solutes than the octanol/water partition coefficient. Therefore, the observed rate of penetration will depend not only on the solubility in the cuticle/waxes, which depends on the lipophilicity of the compound, but also on the rate of evaporation of solvents. After evaporation, the penetration of AI and accelerator surfactants proceeds from surface waxes and/or formulation residues, and the changing concentrations in cuticular waxes and the cuticle influence the rates of penetration, rendering interpretation difficult.

3.2 Surfactant penetration in the absence and presence of solutes

Since the amounts of active ingredient and surfactant are often of the same magnitude we measured [^{14}C]C12E8 penetration under extreme conditions with more than a 20-fold excess of methylglucose, phenylurea or WL 110547. Surfactant penetration alone or in the presence of methylglucose was very rapid and after 8h about 75% of the amount applied to pear cuticles had penetrated (Fig 2). WL 110547 slightly decreased penetration, but this effect occurred

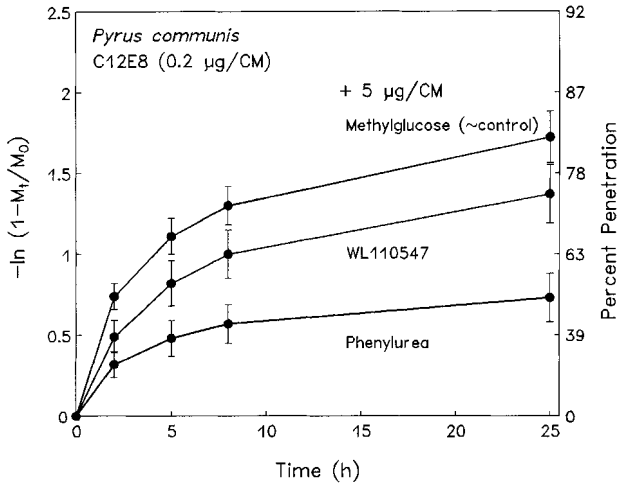

Figure 2. Time course of penetration of C12E8 (0.04g litre^{-1} in a 5-µl droplet) across leaf cuticles at 25°C and 55% RH in presence of excess amounts (1g litre^{-1}) of methylglucose, phenylurea, and WL 110547 (Means of 10 CM with confidence intervals). Penetration of C12E8 in presence of methylglucose was equal to C12E8 penetration alone (control).

only immediately after application, because later (after the first sample at 2h) rates of penetration (slopes) were identical to those of the control. The decrease in penetration after one day was about 10% and a similar effect was observed by Stock and Holloway[3] and Stock *et al*[29] who reported a decrease of surfactant (C13E6) uptake into bean leaves of 15 to 60% in the presence of various amounts of WL 110547.

In contrast to the slight initial effect of WL 110547 and the lack of any effect of methylglucose, an excess amount of phenylurea decreased rates of penetration immediately after application up to the end of the experiment. After one day the amount of [^{14}C]C12E8 penetrated in the presence of phenylurea was about 31% lower than for the control. The negative effect of phenylurea on penetration of C12E8 across *Pyrus* CM agrees with the results obtained for its effects on the penetration of C12E16 into leaves of other species.[29]

We suggest that the effect of phenylurea is related to the free amide group, since even more inhibiting effects on surfactant penetration were observed for an excess amount of urea (Baur, unpublished results). It is known that urea interacts with ethoxy groups and slightly with aliphatic chains and it is sometimes included in pure surfactants to solidify them for better transport properties.[30] The formation of adducts of urea with ethoxylated lauryl alcohol (C12Ex) needs only two oxyethylene groups ($x = 2$). The urea molecules are bound by hydrogen bounds between the oxygen atoms of the oxyethylene groups and the amide groups.[30] Urea and thiourea associate also with pure polyethylene glycols with very high relative molecular mass (up to 4×10^6) to form solid complexes.[31] Inhibition of surfactant penetration due to solidification was also found for C12E8 in the presence of the inorganic salts $CaCl_2$ and $MgCl_2$ but this effect decreased with an increase in humidity.[24]

We have recently shown that [^{14}C]C12E8 penetrates cuticles of various species rapidly (*Citrus aurantium, Ilex paraguariensis, Malus baccata, Malus domestica, Pyrus communis*)[24] and similar observations were obtained with *Avena fatua, Triticum aestivum*, and *Vicia faba*.[29,32] Although differences in the time course of penetration existed among species, penetration after one day was at least 63% (*Citrus aurantium*). Penetration was most rapid with *Pyrus* CM (>80% within one day). Rates of penetration increased immediately after application to a maximum value immediately after complete evaporation of water and decreased thereafter. Humidity had almost no effect, while addition of non-labelled C12E8 or GP C-050 even increased the rates of penetration, since the mobility of C12E8 was increased by addition of the nonlabelled surfactants.[24]

It was mentioned above that, owing to low cuticle/surfactant partition coefficients, penetration of surfactant is a prerequisite for quantitative penetration of solutes. Only if the surfactant reservoir on the surface is depleted (or very low) can the solvent properties of surfactants that decrease partitioning into the cuticle be overcome.

3.3 Mobility effects of EFA surfactants on polymer matrix membranes

It has been shown repeatedly that surfactants affect the mobility of AI in cuticles and that this results from interactions with the cutin/wax composite. Since the increase of mobility is due to increased chain mobility, it is possible that surfactants act similarly by swelling the (cutin) polymer matrix. In *Citrus* MX the mean effect of GP C-050 on mobility of WL 110547 was about 5 (Figs 3C and D). The equilibrium sorption of GP C-050 in the MX was rapid. Maximum effects (constant slopes) with GP C-050 were obtained within one hour, while, for CM, effects did not reach a maximum until four days (Figs 3A and C). This is also indicated by the values for the intercept which was high (6.86) for CM while with MX the theoretical value of 1 (no effect) or below was obtained (Figs 3B and D). In previous work[19] it was found that both monodisperse and polydisperse surfactants in aqueous solutions may need days before maximum effects are obtained. Since the initial rate constants (k^*) were much higher than with CM, this corresponds to a more than 200-fold higher slope in the response plot effect versus $1/k^*$ (compare Figs 3B and D).

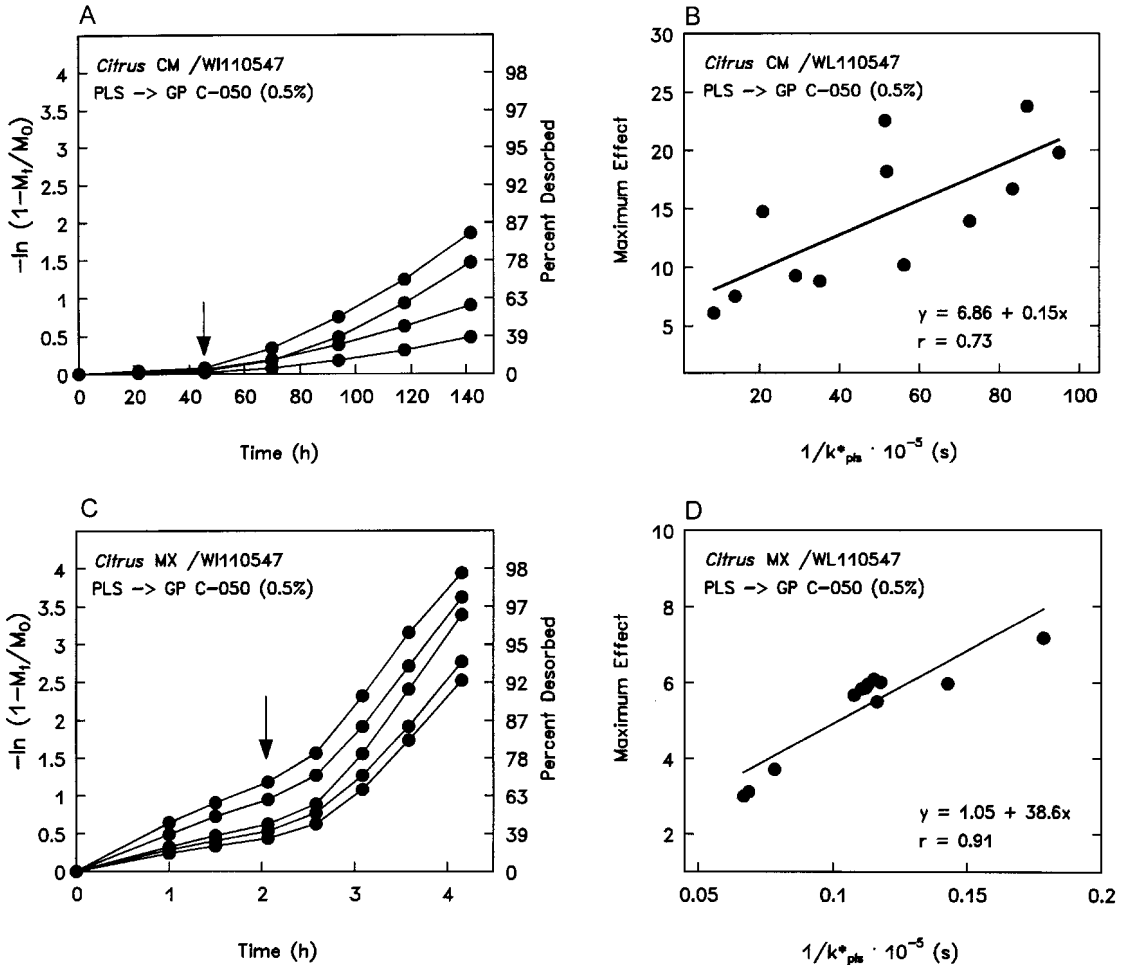

Figure 3. Time course of desorption of WL 110547 from (A) *Citrus* CM and (C) MX with PLS or (indicated by the arrow) an aqueous solution of GP C-050 surfactant. The lines represent examples for the desorption from individual CM. The graphs on the right side show the dependence of the effect of GP C-050 on initial rate constants of desorption of WL 110547 from (B) individual CM and (D) MX.

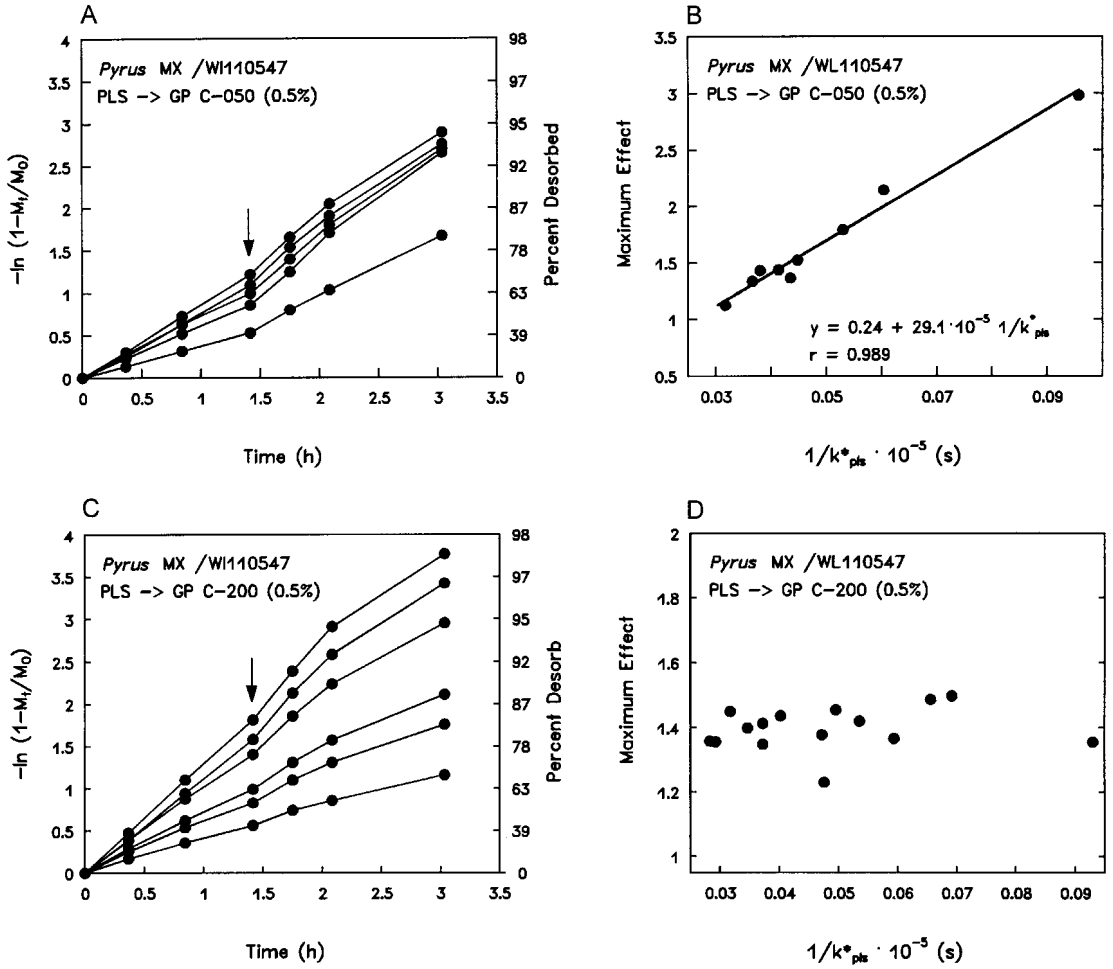

Figure 4. Time course of desorption of WL 110547 from *Pyrus* MX with PLS or (indicated by the arrow) aqueous solutions of (A) GP C-050 and (C) GP C-200 surfactants. The lines represent examples for the desorption from individual CM. The graphs on the right side show the dependence of the effect of (B) GP C-050 and (D) GP C-200 surfactants on initial rate constants of desorption of WL 110547 from individual CM.

Similar results were obtained with GP C-050 on the more open *Pyrus* MX (Figs 4A and B). However, the effect was at a maximum immediately (first sample after 20 min) after application of the surfactant and with some MX it decreased thereafter. The response plot showed good linearity, and the slope of 29 was much higher than with *Citrus* CM (0.15) but similar to that obtained for *Citrus* MX (38.6). The mean effect on *Pyrus* MX (~2-fold) was smaller than with the tighter *Citrus* MX. Even Genapol C-200 increased the mobility of WL 110547 in *Pyrus* MX (Figs 4C and D) though the effect did not vary with initial rate constants but had a similar value of 1.4 for all membranes. With *Pyrus* CM no effect and actually a decrease (effect smaller than 1) in mobility of WL 110547 was observed in previous studies.[19]

These data show that mobility effects of surfactants observed with CM may result from a considerable degree of interaction of the surfactant with the polymer matrix or cutin. Since the transport limiting barrier is within the cuticle and diffusion occurs in amorphous wax and cutin, this effect can add to the surfactant-induced increases of wax fluidity which has been found with monodisperse ethoxylated fatty alcohols for isolated and reconstituted waxes.[33]

3.4 Reversibility of surfactant effects on solute mobility

Surfactant effects on mobility in CM depend on the sorption of a certain amount of surfactant in the cuticle.[34–36] This sorption could cause irreversible changes in the cutin/wax composite. Therefore we tested the time course of desorption into receiver solutions containing first PLS, then surfactant and then finally PLS again. Desorption with surfactant was limited to one to two days to avoid complete depletion of the radiolabelled solutes.

For desorption of bifenox from both *Citrus* CM and *Melicoccus* CM, the increase in mobility induced by polydisperse GP C-050 and monodisperse C12E6 decreased immediately after being replaced by PLS (Figs 5A and B). In these experiments the surfactant effect was not measured until reaching a maximum in order to avoid depletion of the radioactivity which could influence the rates of desorption during the second run with PLS. Desorption of bifenox from *Melicoccus* CM with C12E6 increased its mobility about 18-fold and then decreased after three days' desorption with PLS to 1.88 (±0.35) (95% CI) fold. Similarly, mobility of bifenox in *Citrus* CM increased in the presence of GP C-050 by about 20-fold and

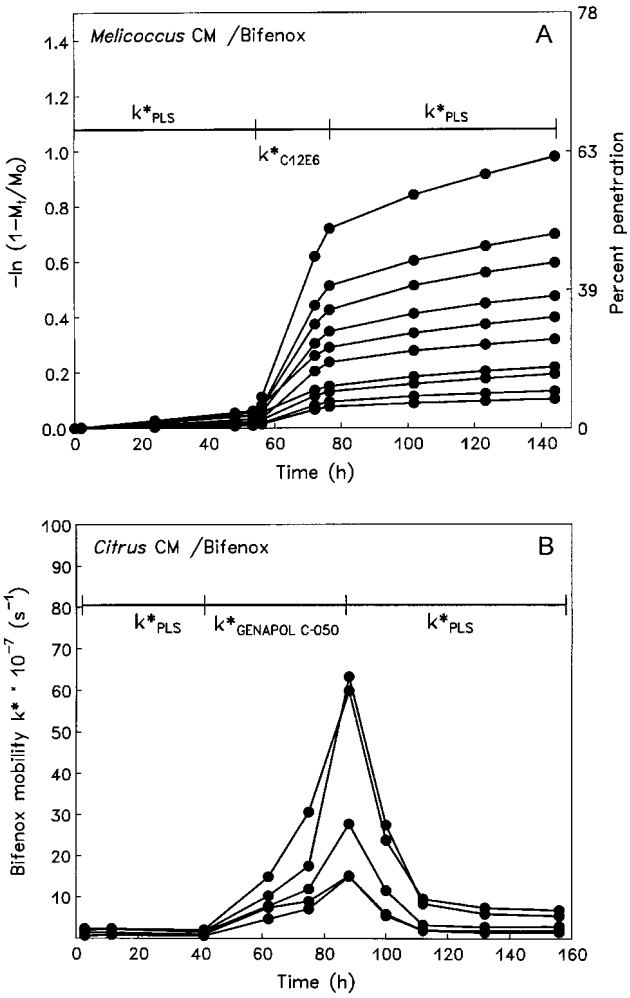

Figure 5. Reversibility of the effect (A) of GP C-050 on the time courses of desorption of bifenox from *Melicoccus* CM and (B) of C12E6 on the mobility (slopes of desorption plot) of bifenox in *Citrus* CM. Desorption of the same set of CM was started with PLS followed by desorption with surfactant for one to two days and again with PLS for three days (see text).

decreased after three days' desorption with PLS to 2.22 (±1.25) of the initial value with PLS before desorption with surfactant.

The effect was almost completely reversible and the decrease seems to depend only on the velocity of extraction of sorbed surfactant with PLS. Owing to the good sorptive properties of PLS it serves as a sink for both bifenox and surfactant, keeping the concentrations in the aqueous phase zero. The decrease of bifenox mobility (Fig 5B) after beginning the second desorption with PLS reflects the desorption of surfactant from the CM. Desorption of surfactant is not expected to follow simple first-order kinetics, since the actual concentration of surfactant influences its own mobility as well.[24,36] Thus, immediately after starting desorption with PLS, rates of desorption of surfactant are highest but they will decrease at low surfactant concentration since the mobility of such large molecules is very low. However, this differs between species, and, for example, with pear CM and with monodisperse surfactants like C8E4 mobility was often even lower after surfactant treatment than before, ie completely reversible. We interpret this reversibility by the dominance of surfactant effects on segmental motion of polymethylene chains, while surfactant-induced phase transitions in, or removal of substantial amount of waxes from, the limiting skin are less probable (see below). The crystallinity of waxes seems to be unchanged by surfactants.[33] These results also show that effects of surfactants on cuticular transport properties depend on the presence of surfactant in the cuticle and underline the importance of simultaneous penetration of active ingredient and accelerator surfactant.[3,4]

With accelerator surfactants the effect on partition coefficients is also temporary, since the surface phase will change due to penetration of surfactant (Fig 2). In contrast, surfactants like Tween 80 which do not penetrate the cuticle may affect partitioning until they are removed from the surface by rain or by other processes like (bio) chemical degradation.

3.5 Temperature dependence of surfactant effects on solute mobility

The above results of surfactant effects relate to a temperature of 25 °C. For any compound or plant species, the maximum effect depends on the maximum equilibrium amount of surfactant sorbed from the aqueous solution.[15,19] Using aqueous solutions the concentration of surfactant in the CM does not change after equilibrium has been obtained. This is a necessary condition for investigating the effect of temperature on mobility in the presence of surfactant. The greatest effects at 25 °C were obtained for aqueous solutions of GP C-050, which has the lowest average number of ethoxy groups. Therefore temperature dependence of surfactant effects was studied with GP C-050 and for comparison also with C8E3, C8E4 and C12E6 as well as with the accelerator tributylphosphate which is not surface-active.

Solute diffusion in the cuticle depends strongly on temperature, as shown by the slopes of the Arrhenius plots (Fig 6) for all compounds and species in the absence of surfactant.[17] The temperature dependence of bifenox mobility in CM of *Stephanotis floribunda* decreased drastically in the presence of the very effective accelerator tributylphosphate, as indicated by the decrease in slope (Fig 6A). At 15 °C the mobility of bifenox was about 55-fold (arithmetic mean) higher in the presence of tributylphosphate. The slopes in the Arrhenius plots are proportional to the activation energy E_D for the diffusion of bifenox, which decreased from 130 kJ mol^{-1} in the absence of tributylphosphate to only 18 kJ mol^{-1} in its presence. This suggests that the cuticle swells, ie takes up a substantial amount of tributylphosphate, since the value of 18 kJ mol^{-1} is a value more typical for a liquid phase. Assuming a lack of specific pores or channels in the transport limiting skin of cuticles, which have never been demonstrated so far, this absorption results in swelling of the cuticle as observed for many synthetic polymers[37] (see below) and increased fluidity

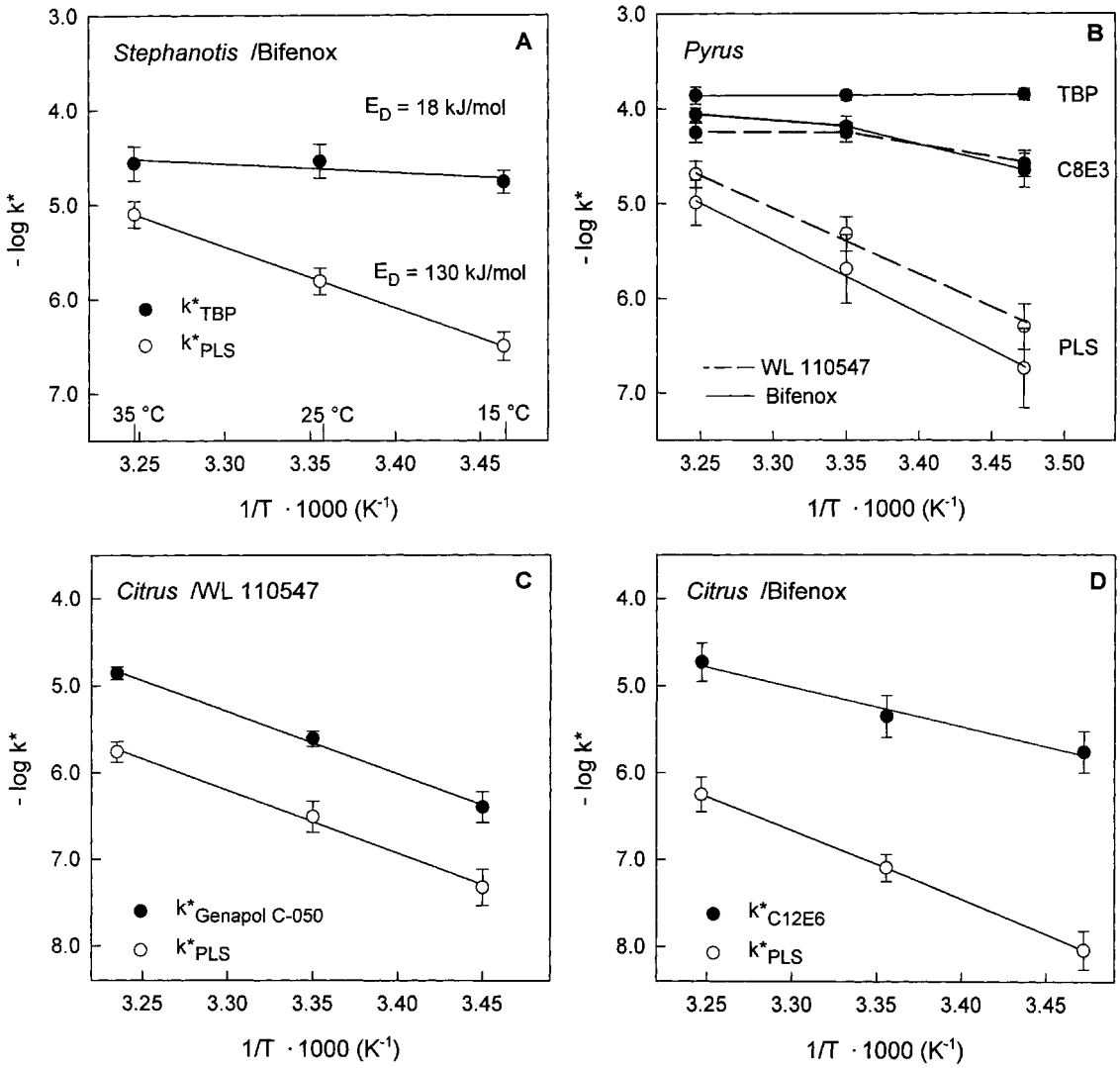

Figure 6. Arrhenius graphs for the mobility of organic solutes in cuticles in (○) absence or (●) presence of monodisperse (25 mM) and polydisperse (0.5% m/m) ethoxylated fatty alcohol surfactants and tributylphosphate (0.5% m/m in PLS). Data for (B) *Pyrus* /WL 110547 and *Pyrus*/Bifenox and (C) *Citrus* /WL 110547 were obtained from two consecutive runs with the same CM by measuring mobility first with PLS (control) and after cooling with surfactant. Data for (A) *Stephanotis* /Bifenox and (D) *Citrus* /Bifenox were obtained from different sets of CM for control (desorption with PLS) and treatment (desorption with TBP or C12E6). Desorption of bifenox from *Citrus* CM was started at 35 °C followed by 25 and 15 °C while in all other experiments desorption started at 15 °C and temperature was increased to the indicated values thereafter. Arithmetic mean values of log *k** for five to 16 CM tested with 95% confidence were plotted.

of waxes.[33] A similar effect of tributylphosphate was observed with pear CM where the mobility of bifenox was very high and almost no temperature dependence could be observed (Fig 6B). At 15 °C mobility was increased by as much as 275-fold by tributylphosphate.

A large increase of mobility in pear CM was also observed with C8E3, 123-fold for bifenox and 52-fold for WL 110547 (Fig 6B) at 15 °C. Slopes for both bifenox and WL 110547 decreased in the presence of C8E3, though to a lesser extent than with TBP. The value at 35 °C is uncertain, since almost 90% of the radiolabelled compounds (bifenox and WL 110547) had been desorbed after the last sample at 25 °C in the presence of C8E3 had been taken. Although we are not able to calculate correct values for E_D in the presence of C8E3, the values are at least 100 kJ mol^{-1} lower (from the values at 15 and 25 °C) than those in its absence (E_D was 149 kJ mol^{-1} for bifenox and 137 kJ mol^{-1} for WL 110547) which is in accord with the low value of 17 kJ mol^{-1} for the mobility of WL 110547 in pear CM in the presence of C8E4, as previously reported.[36]

Interestingly, with GP C-050 (Fig 6C), the Arrhenius plot is parallel to that of desorption into PLS with the surfactant effect on mobility of WL 110547 in *Citrus* CM at each of the temperatures examined being rather constant with values of 9.5 (at 16.5 °C), 8.4 (25 °C), and 9.3 (35.5 °C). These values may suggest that the polydisperse surfactant increases mobility by a constant factor through the temperature range studied. However, equilibrium between the cuticle and the aqueous micellar surfactant solution may not have been established. For example, the maximum effect of GP C-050 on WL 110547 mobility after four days was 15.2 (Fig 3A) which is significantly

higher than the value of 8.4 at 25 °C (from Fig 6C) in this experiment. At 16.5 °C the rate constants for WL 110547 with GP C-050 were unlikely to be maximal and would be expected to be much higher at complete equilibriation. Even with C8E4 the value for the mobility of WL 110547 in *Citrus* CM at 15 °C was rather low due to non-equilibrium sorption of C8E4 in another experiment (data not shown) and a similar result was obtained for the same species and 2,4-D.[36] The reason for the long initial periods at lower temperatures is probably related to the large molar volumes of C8E3, C8E4, C12E6, and monomers of GP C-050 ($>266\,cm^3\,mol^{-1}$) which cause a low solute mobility at low surfactant concentrations (see also Section 3.4). During sorption of surfactant, solute mobility in the cuticle increases, since these surfactants are accelerators, and this increases mobility for all compounds sorbed, including the surfactants themselves. At lower temperatures, sorption and rates of diffusion of both accelerators and AI will also be slow. Notably, at the highest temperature of 35 °C, the mobilities of WL 110547 in *Citrus* CM in the presence of C8E4 ($-\log k^* = 4.88$) and GP C-050 ($-\log k^* = 4.86$) were identical.

To avoid the experimental error due to the low velocity of surfactant penetration into *Citrus* CM at low temperatures from aqueous solution, one experiment with monodisperse C12E6 (the average formula of polydisperse GP C-050 is C12.5E5.8) was started by desorption of bifenox at 35 °C followed by desorption at lower temperatures (Fig 6D). In this case the value of k^* at 15 °C is not low and an activation energy of diffusion of $87\,kJ\,mol^{-1}$ in the presence of C12E6 is calculated. This value is significantly lower than the value of $160\,kJ\,mol^{-1}$ for the desorption with PLS (control). Since mobilities of solutes are similar in the presence of accelerator surfactants (see next paragraph) a similar result is expected for WL 110547.

The Arrhenius graph for *Pyrus* (Fig 6B) shows that the accelerating effect of C8E3 at all temperatures is greater for bifenox than for WL 110547. The reason for this difference is that the mobility of bifenox is much lower in the absence of C8E3, while in its presence mobilities of both compounds are similar. The same holds for the greater effect of C12E6 on desorption of bifenox compared to that of GP C-050 (≈C12E6) on WL 110547. An analogous result was obtained for the effect of C8E4 on the mobility of bifenox and 2,4-D in *Citrus* CM.[36] We have previously shown[19] that the surfactant effect (at 25 °C) is larger with compounds having high molar volumes and this study indicates that the effect will also decrease with increasing temperature or will be greater at lower temperatures, respectively. For example, the mobilities of bifenox and WL 110547 in *Pyrus* and *Stephanotis* at 15 or 25 °C in the presence of C8E3 or tributylphosphate are as high as the mobilities without accelerator at about 60 °C. This result is reasonable, since surfactants and an increase in temperature act similarly by a decrease of the cohesive energy of polymer chains, which leads to higher segmental mobility of the polymethylene chains of the wax/cutin composite similar to the behaviour of synthetic polymers.[37] An increase in fluidity after sorption of EFA surfactants was shown for reconstituted waxes.[33]

Our studies on the effect of accelerator surfactants on activation energies of diffusion were obtained with aqueous solutions. Under these conditions the uptake of surfactant molecules is related to the concentration of surfactant monomers and is probably slower and to a lesser extent than under practical conditions where uptake proceeds from a highly concentrated formulation residue. The observed reductions of E_D are therefore probably conservative values. We expect that the effect may be even greater if a pure surfactant residue is present on the surface.

3.6 Variability among individual cuticles as affected by surfactants

By comparing solute mobilities in CM of several species in the absence and presence of various accelerators it was shown recently that accelerators not only increase mobility but simultaneously drastically reduce the variability among individual cuticles.[38] The same effect was found for several species when the temperature was increased.[17] Our data for the mobility of WL 110547 in *Citrus* cuticles enables paired observations (the same CM were used) for both the effects of increasing temperature and of sorption of GP C-050 in CM on variability and in addition, these data can also be compared with MX with or without GP C-050. The data in Fig 7 show that both treatments successively reduced coefficients of variation from about 92% without GP C-050 at 15 °C to 26% with the surfactant at 35 °C in a systematic manner. Coefficients of variation for MX at 25 °C were lower, about 27%, which is much lower than the value for

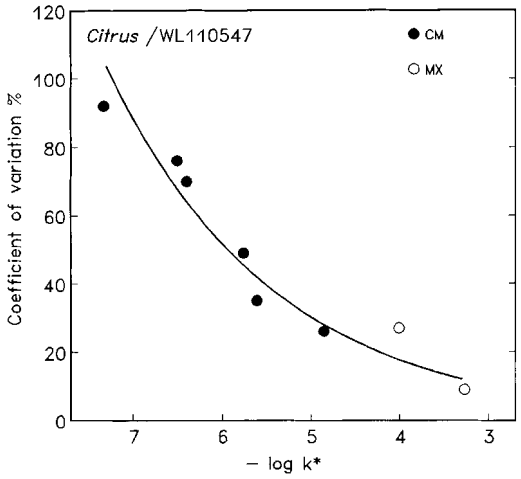

Figure 7. Coefficients of variation at increasing values of rate constants of desorption (log k^*) due to increasing temperatures and/or the presence of GP C-050 with (●) *Citrus* CM and (○) MX. Coefficients of variation were calculated from data in Fig 6C (CM) and Fig 3C ($n = 12$ for CM and MX). Data for CM resemble paired observations as they were successively exposed to higher temperatures and/or surfactant. Data are fitted by an exponential curve.

CM at this temperature (76%) but similar to the value for CM in presence of GP C-050 at 35 °C. Variability of MX was reduced further by GP C-050 to a value of about 9% (Fig 7). These results indicate that the observed surfactant effects reflect interactions with the wax/cutin composite of the cuticle and without wax (MX) both effects on mobility and variability are accordingly much lower, suggesting that the variability has its origins in the wax component and not in the polymerised cutin.

4 CONCLUSIONS

Surfactants can influence rates of cuticular penetration by changing the diffusivity in the cuticle and by changing driving force. Many active ingredients are lipophilic and they will be dissolved in surface waxes of many species during droplet drying. Dissolution in surface waxes does not mean that the compounds are necessarily transported through the whole cuticle but surfactants can improve the rates of penetration beneath the surface waxes by increasing the mobility in the transport limiting skin of the cuticle.

In contrast to this effect, the good solvent properties of ethoxylated fatty alcohol surfactants simultaneously can cause the compounds to partition poorly from the surfactant residue into the cuticle. Since the partition coefficients are low, the driving force will be decreased. Therefore, for lipophilic compounds it is appropriate to keep the concentration of the active ingredient in the formulation residue high by choosing low amounts of surfactant if the surfactant itself does not penetrate the cuticle. A high concentration can also be used if the surfactant itself penetrates the cuticle rapidly, as is the case with the Genapol C-050 surfactant used in this study.

A good formulation for polar compounds has other requirements. Since the solubility of these compounds in surface wax is low, a solid deposit is likely to be formed in the absence of any adjuvants or with adjuvants having insufficient solvent power and this results in poor penetration. In this case, the main effect of surfactant residues is to avoid precipitation or to redissolve the compounds, possibly by additional action as humectant, and produce a liquid phase to enable diffusion into the cuticle. This is a prerequisite for an additional increase of rates of penetration by the effects of (perhaps the same) surfactants on mobility.

Surfactants which penetrate the cuticle affect mobility and partition coefficient in a time-dependent manner and it will be important to match the rates of penetration of adjuvant and AI through a temporarily modified cuticle to achieve maximal rates of AI uptake and efficiency.

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